International Journal of Agricultural Science and Research (IJASR)
ISSN(P): 2250-0057; ISSN(E): 2321-0087
Vol. 5, Issue 4, Aug 2015, 139-148

EVALUATION OF GENETIC DIVERSITY OF THE IMPORTANT MEDICINAL PLANT ALOE (*ALOE BARBADENSIS MILLER*) USING RAPD AND ISSR MARKERS

PUSHPA DEORE[1] & S. SAMANTARAY[2]

[1]Department of Agricultural Botany, Anand Agricultural University, Gujarat, India

[2]Directorate of Medicinal and Aromatic Plants Research, Boriavi, Anand, Gujarat, India

ABSTRACT

Aloe barbadensis Miller is an ayurvedic, xerophytic, succulent, medicinal plant commonly known as *Aloe vera* and it is used worldwide in drug and cosmetic industry. In the present investigation, genetic diversity in 38 elite accessions of *Aloe vera*, collected from different geographical regions of India, were evaluated using Random Amplified Polymorphic DNA (RAPD) and Inter Simple Sequence Repeat (ISSR) markers. Molecular polymorphism was 85.39% with 14 RAPD primers and 72.5% with 06 ISSR primers indicating high level of genetic variation among the accessions and the mantel test revealed positive correlation between the two marker systems. Dendrogram was constructed based on pair wise genetic similarities and three-dimensional principal coordinate analysis using data from RAPD and ISSR marker systems showed similar clustering pattern and separated accessions into two major groups. Both the markers techniques (RAPD and ISSR) have been shown to be useful in detecting small genetic variations within and among *Aloe vera* populations.

KEYWORDS: *A. barbadensis*, Genetic Variability, ISSR, Molecular Marker, RAPD

INTRODUCTION

Aloe barbadensis Miller is an ancient, perennial plant found in tropical and subtropical areas, particularly in South Africa and Arabia. The name *Aloe vera* was derived from the Arabic word ‘alloeh’ meaning ‘shining bitter substance’. *Aloe* species are generally recognized by their rosettes and propagated worldwide for pharmacological and cosmetic industries (Bhaludra *et al*., 2013). Although about 360 species of *A. vera* have been reported, only *Aloe barbadensis* Miller commonly referred as “Ghrith Kumari” in Hindi and has become naturalized almost in all regions of India (Klein and Penneys, 1988). *Aloe vera* is the most important among the *Aloe* species and it can be found growing in the tropical climates of the South America, Southern United States, Central America, India, Australia, the Caribbean and Africa. Traditionally, *A. vera* is grown as an ornamental plant and used in herbal medicine. However, the commercial use of the *Aloe vera* plant was in the production of a latex (yellowish sap) substance called aloin used for many years as a laxative and purgative ingredient (Boudreau and Beland, 2006). *Aloe vera* gel has been used in cosmetics like moisturizers, sunscreens, soaps and shampoos. Aloe juice has been shown to lower cholesterol and triglycerides while demonstrating anti diabetic activity. Various scientific studies on aloe gel have demonstrated for its anti-inflammatory, wound healing, immune modulating, analgesic, anti-tumor activities, anti-bacterial, antifungal and antiviral properties (Manvitha and Bidya, 2014). It accelerates, regulates the metabolism and purifies the toxins from human body. In order to improve the medicinal values of *Aloe vera* and also to fill the gap between demand and supply of elite plant material, there is a need to conserve this species for sustainable use in future. The success of any genetic conservation and breeding

program depends largely on the identification of the amount and distribution of genetic diversity in the gene pool of the concerned plant. Knowledge on the genetic diversity and relationships among plant varieties is important to recognize gene pools, to identify gaps in germplasm collection and to develop effective conservation and management strategies. Since morpho-chemical characters are dependent on age and environment, it is essential to characterize this medicinally and economically important genus genetically (Nayanakantha *et al*., 2010). In recent years, DNA-based molecular markers have been used to assess the genetic diversity among the germplasm in many plant species. DNA based molecular markers have the advantage of being free from environmental modulations. In *A. vera*, RAPD and AFLP markers have been used to study the genetic diversity among different *Aloe* species (Darokar *et al*., 2003; Shioda *et al*., 2003) and with these RAPD markers identified three species of Aloe (*Aloe vera*, *A. arborescence*, *Aloe ferox*). Nejatzadeh-Barandozi *etal*. (2012) and Tripathi *et al*. (2011) has used RAPD and AFLP markers to assess genetic diversity among aloe accessions obtained from different geographical regions of Madhya Pradesh in India and Iran, respectively. In this current investigation, genetic diversity was evaluated in *Aloe barbadensis* Miller germplasm obtained from different geographical regions of India using RAPD and ISSR primers keeping in view the low development and running costs per data point besides detection of genome-wide variation.

MATERIAL AND METHODS

Collection of Plant Material

A germplasm collection of thirty eight accessions of *A. barbadensis* was organized from different parts of India, Gujarat, Rajasthan, Madhya Pradesh and NBPGR, New Delhi and maintained under uniform growth conditions at DMAPR, Anand, Gujarat. The details of accessions used in the study are presented in Table 1.

DNA Extraction

Total genomic DNA was extracted from younger leaves of 38 *Aloe vera* accessions by following the standard CTAB method with minor modifications (Doyle and Doyle, 1990). Two grams of leaves were ground in liquid nitrogen, then homogenized in 10 mL of extraction buffer (4% CTAB, 20 mM EDTA, 2% PVP, 1.4 M NaCl, 100 mM Tris-HCl pH 8.0 and 1% β mercapto ethanol) and incubated at 65°C for 30 min. The supernatant was twice extracted with chloroform: isoamylalcohol (24:1, v/v) and treated with 2 µL RNase (100 mg/mL), incubated at 37°C for 1 h. The DNA was precipitated with isopropanol and washed twice with 70% ethanol. The pelleted DNA was air dried and re suspended in 500 µL of sterile Millipore water and finally all DNA samples were diluted to get 50 ng/µL and were stored at -20°C for use in RAPD and ISSR assay.

ISSR Amplification

A total of twelve six ISSR primers were used for the analysis (Table 2). PCR amplification was performed with minor modifications (Zeitkiewicz *et al*. 1994). ISSR amplification reactions were carried out in 25µl reaction volumes containing 2.5 µl of 10X assay buffer (100 mMTris-Cl; pH 8.3, 500mMKCl, 15mM $MgCl_2$), 0.2 mM of each dNTPs (dATP, dCTP, dGTP and dTTP), 5 pg of primer, 1.0 unit of Taq DNA polymerase and 25ng template DNA. The amplification reaction was carried out in a DNA Thermal Cycler (Eppendorf AG, Hamburg, Germany) programmed for 43 cycles using the following amplification profile: initial denaturation of template DNA at 94°C for 5 minutes followed by 43 amplification cycles of denaturation at 92°C for 1 minute primer annealing at 50–56°C (ISSR) for 1 minute and elongation at 72°C for 2 minutes followed by a final extension step at 72°C for 7 minutes. The amplified products were

electrophoresed at 120 V on a 1.5% agarose gel in 1XTAE buffer using 100-bp ladder (Fermentas, USA) as the molecular weight standard.

RAPD Amplification

PCR amplification (Williams *et al.* 1990) was performed with arbitrary decamer primers obtained from Operon Technologies, Alameda, USA (Table 3). Amplifications were performed in 25µl reaction volumes containing 2.5µl of 10X assay buffer (100mMTris-Cl; pH 8.3, 500mMKCl, 15mM $MgCl_2$), 0.2mM of each dNTPs (dATP, dCTP, dGTP and dTTP), 5pg of primer, 1.0 unit of Taq DNA polymerase and 30ng template DNA. The amplification reaction was carried out in a DNA Thermal Cycler (Eppendorf AG, Hamburg, Germany) programmed for 43 cycles using the following amplification profile: initial denaturation of template DNA at 94°C for 5 minutes followed by 43 amplification cycles of denaturation at 92°C for 1 minute primer annealing at 37°C for 1 minute and elongation at 72°C for 2 minutes followed by a final extension step at 72°C for 7 minutes. After completion of amplifications, PCR product was resolved on 1.5% agarose gel in 1×TAE buffer by electrophoresis at 120 V for 1h and 20 minutes then visualized with under the UV light and photographed in a gel documentation system (Syngene, UK). The sizes of the amplicons were determined by comparing them with the 100-bp ladders (Fermentas, USA). The entire process was repeated at least twice to confirm the reproducibility.

Statistical Analysis

DNA fingerprints were scored for the presence (1) or absence (0) of bands of various molecular weight sizes in the form of binary matrix. Data were analyzed to obtain Jaccard's coefficients among the isolates by using NTSYS-pc (version 2.02c, Rohlf 1993). The SIMQUAL program was used to calculate Jaccard's coefficients (Jaccard 1908), which are common estimator of genetic identity and were calculated as follows:

Jaccard's coefficient = $N_{AB}/(N_{AB}+N_A+N_B)$

Where N_{AB} is the number of bands shared by samples; N_A represents amplified fragments in sample A, and N_B represents fragments in sample B. Similarity matrices based on these indices were calculated. Correlation between the two matrices obtained with two marker types (ISSR and RAPD) was estimated by means of Mantel (1967) test. Coefficient correlation (r) is provided one measure of relatedness between the two matrices. Similarity matrices were utilized to construct the unweighted pair-group method with arithmetic average (UPGMA) dendrograms. In order to estimate the congruence among dendrograms, cophenetic matrices for each marker were computed and compared using Mantle test. Principal coordinate analysis was performed in order to highlight the resolving power of the ordination.

The ability of the most informative primer pairs to differentiate between the genotypes was assessed by calculating their resolving power according to Prevost and Wilkinson (1999) using Rp=ΣIB [IB (band informativeness) = 1- [2 × (0.5 – P)], P is the proportion of the 38 accessions (Aloe accessions analyzed) containing the band. The Primer Index was calculated from the polymorphic Index. The Polymorphic Index (PIC) was calculated as PIC = Σ P2i, Pi is the band frequency of the ith allele (Smith *et al.*, 1997). Here, the PIC was considered to be 1-p2-q2, where p is the band frequency and q is no band frequency (Ghilslain et al., 1999). PIC value was then used to calculate the RAPD and ISSR Primer Index (PI). PI is the sum of the PIC of all the markers amplified by the same primer.

RESULTS

Both the marker systems being employed to assess the genetic diversity in *Aloe vera* accessions were quite informative and were able to generate adequate polymorphism shown in Figure 1 and 2 among the *A. barbadensis* accessions tested.

ISSR Band Pattern

The PCR amplification using ISSR primers gave rise to reproducible amplification products. ISSR primers produced different numbers of DNA fragments, depending upon their simple sequence repeat motif. The 06 primers produced 40 bands across 38 genotypes, of which 29 were polymorphic, accounting for 72.5% polymorphism (Table 2). The number of bands ranged from five [$(CA)_8$AT) and $(GA)_7$RC]to eight [$(CT)_8$TG),$(CA)_8$GT and $(CT)_9$G] and varied in size from150 to 1950bp. Average number of bands and polymorphic bands per primer were 6.67 and 5, respectively. Percentage polymorphism ranged between 60 [$(GA)_7$RC)] and 87.5 [$(CT)_8$TG)], with an average of 74.028 and Resolving Power (RP) of the primers varied from 3.579 to 12.474 while the primer index varied from 0.344 to 0.4944. Best Resolving Power was observed in the Primer $(CA)_8$GT. The maximum ISSR Primer Index (PI) (0.4944) was observed with the primer $(CT)_8$TG and the minimum 0.344 with the primer (CA)8GT. Primer $(CT)_8$TG showed maximum number of polymorphic loci (87.50). A dendrogram based on UPGMA analysis with ISSR data is shown in Figure 3b. Jaccard's similarity coefficient ranged from 0.41 to 0.97. The 38 genotypes were grouped into two main clusters.

RAPD Band Pattern

PCR amplification of DNA, using 14 primers for RAPD analysis (figure 2), produced 89 DNA fragments that could be scored in all genotypes. All the selected primers amplified DNA fragments across the 38 genotypes studied, with the number of amplified fragment varying from three (OPJ20) to eleven (OPJ11), with size ranging from 190 to>3000bp. Of the 89 amplified bands, 76 were polymorphic, with an average of 5.429 polymorphic bands per primer. Percent polymorphism ranged from 66.67 (OPJ18) to 100 (OPJ13), with an average percentage polymorphism of 82.76. The Resolving Power (RP) of the primers varied from 3.842 to 14.947, while the primer index varied from 0.185 to 0.500. Best Resolving Power was observed in the Primer OPJ 11. The maximum RAPD Primer Index (RPI) (0.500) was observed with the primer OPJ 20 and OPN 16 and the minimum (0.185) was in OPA 09 (Table 3). A dendrogram based on UPGMA analysis grouped the 38 genotypes into two main clusters, with Jaccard's similarity coefficient ranging from 0.42 to 0.93 (Figure 3a).

Combined (ISSR and RAPD) Data

The genetic similarity matrix data generated using RAPD and ISSR systems were compared. Mantel test for congruence of RAPD and ISSR data matrices indicated a goodness of fit (r = 0.724) indicating good correlation between the two molecular marker systems. Although the two marker systems sampled different segments of the genome, the clustering pattern of the genotypes was almost similar with both the marker systems and most of the accessions were placed in their respective clusters with minor changes.

On the basis of data obtained from RAPD and ISSR markers, a dendrogram was constructed using UPGMA clustering. Dendrogram constructed from combined RAPD and ISSR data segregates the alone accession (12) from rest of the accessions sharing a node at 56% level of similarity. Like RAPD analysis, no 12 accession was also singled out

whereas no 1 and 11 formed a group and all the remaining accessions were distantly related to each other. In subsequent sub clusters accession no 2, 4 and 5 formed a group except accession no3. (In RAPD 2, 3, 4, 5 are in one group). Group VA consisted of four accessions no. 15 collected from Gujarat, 16 collected from Madhya Pradesh, 17 collected from Gujarat and 19 from Gujarat showed 80% genetic similarity.

Principal Coordinate Analysis (PCO) based on genetic similarity showed the relationship among accessions in three dimensional spaces. The PCO analysis based on pooled data of RAPD and ISSR primers grouped the accessions into three groups as given in Figure 3d which is similar to the UPGMA clustering pattern.

DISCUSSIONS

An understanding of the extent of genetic diversity is important for both plant breeding and germplasm collection. In *Aloe vera* traditional methods like horticultural traits are relatively less reliable and inefficient for precise discrimination of closely related genotypes (Nejatzadeh-Barandozi *et al*., 2012). Hence, selection based on genetic information using neutral molecular markers is essential as it is more reliable and consistent. Among different marker systems available, RAPD and ISSR markers became popular in diversity studies because of simplicity, rapid, inexpensive and applicable to any genome without any prior information regarding the genome of the plant.

In the present study, a set of 38 elite *Aloe vera* accessions (Table 1) were analyzed using 89 RAPD and 40 ISSR markers to describe the genetic structure among the accessions. The RAPD primers revealed 85.39% polymorphism with 5.429 polymorphic bands/primer, while ISSR primers revealed 72.5% polymorphism with 5.0 polymorphic bands/primer indicating wide genetic variation among the accessions. ISSR primers detect more polymorphism than RAPD primers because of variability in microsatellite loci due to DNA slippage (Williams *et al*., 1990). The RAPD markers cover the entire genome in coding and non coding regions including repeated or single-copy sequences, while ISSR markers disclose polymorphism from sequences between two microsatellite primer sites (Williams *et al*., 1990; Zietkiewicz *et al*., 1994). The ISSR method has been reported to be more reproducible (Goulao and Oliveira, 2001) and produces more complex marker patterns than the RAPD approach (Chowdhury *et al*., 2002; Parsons *et al*., 1997) reported that is an advantageous when differentiating closely related cultivars. Both the marker techniques provides a useful approach for evaluating genetic differentiation, significantly in those species that are poorly known genetically and are propagated vegetatively like monocot genus in *Musa* (Bhat and Jarret, 1995) and *Lilium* (Haruki *et al*., 1998). Nevertheless, on the basis percent polymorphism (RAPD=85.39 and ISSR=72.5) and average expected gene diversity (RAPD=0.346; ISSR=0.361and similarity matrix, the RAPD markers were marginally more informative than ISSR in the assessment of genetic diversity in *A. barbadensis*. The similar results are reported in *Caldesia grandis*, *Dalbergia sissoo* and *Prunusarmeniaca* (Chen *et al*., 2006; Arif *et al*., 2009; Kumar *et al*., 2003). This may be because of the fact that two marker techniques targeted different portions of the genome. Some studies have shown that RAPD markers are distributed throughout the genome and may be associated with functionally important loci (Penner 1996).

The PCO analysis and dendrogram constructed based on RAPD+ISSR polymorphism showed similar clustering pattern and disclosed predominantly two major clusters (A and B). The similarity values ranging from 45 to 86% indicating that there is a remarkable genetic variation among *Aloe vera* accessions used in the present study. The highest similarity (86%) was recorded between IC285629 and GUJ3 accessions which were collected from Gujarat, India. Recently, AFLP based characterization of *Aloe vera* accessions from different location of Madhya Pradesh, India has reported modest level

of genetic variability (Tripathi *et al*., 2011). This low level of variability could be because of small sample size collected from the limited geographical regions. Similarly, Darokar *et al*. (2003) has also reported the morphological similarity 78.8-99% in *Aloe vera* accessions revealed by RAPD and AFLP analysis. Nayanakantha *et al*. (2010) have reported a good amount of genetic variability among *Aloe vera* accessions based on RAPD analysis.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the financial assistance from Director, DMAPR, Boriavi, and Department of Agricultural Botany, Anand Agricultural University, Gujarat for providing necessary facilities. The authors are also thankful to Dr. (Ms.) K. A. Geetha, Senior Scientist (Plant breeding) for providing the plant materials for this study.

REFERENCES

1. Arif, M., Zaidi, Singh, Y. P., Quazi, M. R. H. and Singh, U. S. 2009. A comparative analysis of ISSR and RAPD markers for study of genetic diversity in Shisham (*Dalbergia sissoo*). *Plant Mol Biol Rep.* 72:488-495.
2. Bhaludra, C. S. S., R. R. Bethapudi, A. C. Murugulla, C. Pullagummi and T. Latha, Venkatesh, k., Bheemagani, A. J., Pudutha, A. and Rani, A. R. 2013. Cultivation, phytochemical studies, biological activities and medicinal uses of *Aloe ferox*, Grandfather of *Aloes* an important amazing medicinal plant. *Int. J. Pharmacol*. 9: 405-415.
3. Bhat, K. V. and Jarret, R. L. 1995. Random amplified polymorphic DNA and genetic diversity in Indian *Musa* germplasm. *Genet. Resour Crop Evol*. 42: 107-118.
4. Boudreau, M. D. and F. A. Beland, 2006. An evaluation of the biological and toxicological properties of *Aloe barbadensis* (miller), *Aloe vera*. J. Environ. Sci. Health Part C: Environ. Carcinog. *Ecotoxicol. Rev*. 24: 103-154.
5. Chen, J. M., Gituru, W. R., Wang, Y. H., Wang, Q. F. 2006. The extent of clonality and genetic diversity in the rare *Caldesiagrandis* (Alismataceae): comparative results for RAPD and ISSR markers. Aquat Bot. 84:301–307.
6. Chowdhury M. A., Vandenberg. B. and Warkentin, T. 2002. Cultivar identification and genetic relationship among selected breeding lines and cultivars in chickpea (*Cicerarietinum* L.). *Euphytica.* 127:317–325.
7. Darokar, M. P., R. Rai, A. K. Gupta, A. K. Shasany, S. Rajkumar, V. Sundaresan and S. P. S. Khanuja. 2003. Molecular assessment of germplasm diversity in *Aloe* spp. using RAPD and AFLP analysis. *J. Med. Arom. Plant Sci*. 25: 354-361.
8. Ghilslain, M., Zhang, D., Fazardo, D., Huamann, Z. and Hismans, R. H. 1999. Marker-assisted sampling of the cultivated Andean Potato *Solanumfureja* collection using RAPD markers. *Genetic Resources and Crop Evolution*. 46: 547-555.
9. Goulao, L. and Oliveira, C. M. 2001. Molecular characterization of cultivars of apple (*Malus domestica* Borkh.) using microsatellite (SSR and ISSR) markers. *Euphytica*. 122:81–89.
10. Haruki, K., T. Hosoki and Y. Nako, 1998. RAPD analysis of *Lilium japonicum* Thunb. native to Shimane and other prefectures (Japan). *J. Japanese Soc. Hort. Sci. (Japan)*. 67: 785-791.
11. Jaccard, P. 1908. Nouvelles researches sur la distribution florale. Bull. Soc. *Vaud. Sci. Nat*. 44: 223-270.
12. Klein, A. D. and N. S. Penneys. 1988. *Aloe vera*. J. Am. Acad. *Dermatol.* 18: 714-720.

13. Kumar, P., Singh, K., Vikal, Y., Randhawa, L. S. and Chahal, G. S. 2003. Genetic diversity studies of elite cotton germplasm lines Using RAPD markers and morphological characters. *Indian J Genet*. 63(1): 5-10.

14. Mantel, N. 1967. The detection of disease clustering and generalized regression approach. *Cancer Res*. 27:209–220

15. Manvitha, K. and B. Bidya. 2014. *Aloe vera*: A wonder plant its history, cultivation and medicinal uses. J. Pharmacogn. *Phytochem*. 2: 85-88.

16. Nayanakantha, N. M. C., B. R. Singh and A. K. Gupta, 2010. Assessment of genetic diversity in Aloe germplasm accessions from India using RAPD and morphological markers. *Ceylon J. Sci. Biol. Sci*. 39: 1-9.

17. Nejatzadeh-Barandozi, F., M. R. Naghavi, M. E. Hassani, Y. Mostofi, A. Mousavi and S. Tahmasebi Enferadi. 2012. Diversity of Iranian Aloe (*Aloe vera* L.) genotypes based on aloenin contents and some morphological traits. *The J. Hortic. Sci. Biotechnol*. 87: 673-677.

18. Parsons, B. J., Newbury, H. J., Jackson, M. T. and Ford-Lloyd B. V. 1997. Contrasting genetic diversity relationships are revealed in rice (*Oryza sativa*L.) using different marker types. *Mol Breed*. 3:115–125.

19. Penner, G. A. 1996. RAPD analysis of plant genomes. In: Jauhar PP (ed) Methods of genome analysis in plants. CRC, Boca Raton, pp 251–268

20. Rohlf, F. J. 1993. NT-SYS-pc: numerical taxonomy and multivariate analysis system, version 2.11 W. Exteer Software, Setauket.

21. Shioda H, Satoh K, Nagai F, Okubo T, Seto T, Hamano T, Kamimura H, KanI. 2003. Identification of *Aloe* species by random amplified polymorphic DNA (RAPD) analysis. *Shokuhin Eiseigaku Zasshi*. 44: 203-207.

22. SmithC, J. S. C., hin, E. C. L., Shu, H., Smith, O. S., Wall, S. J., Senior, M. L., Mitchell, S. E., Kresovich, S. and Ziegle, J. 1997. An evaluation of the utility of SSR loci as molecular markers in maize (*Zea mays* L.): comparison of data from RFLPs and pedigree, *Theor. And Applied Genetics*. 95: 163-173.

23. Tripathi, N., N. Saini and S. Tiwari. 2011. Assessment of genetic diversity among *Aloe vera* accessions using amplified fragment length polymorphism, *Int. J. Med. Arom. Plants*. 1: 115-121.

24. Williams, J. G. K., Kubelic, A. R., Livak, K. J., Rafalsky, J. A. and Tingey, S. V. 1990. DNA polymorphisms amplified by arbitrary primer are useful as genetic markers. *Nucleic Acid Research*. 18: 6531-6535.

25. Zietkiewicz, E., Rafalski, A. and Labuda, D. 1994. Genome fingerprinting by simple sequence repeat (SSR) anchored polymerase chain reaction amplification. *Genomics*. 20: 176–183.

APPENDICES

Table 1: Details of *A. barbadensis* Accessions Used in Diversity Analysis

Sr. No.	Accession	Place of Collection	Sr. No.	Accession	Place of Collection
01	IC310611	Gujarat	20	NR57	Gujarat
02	IC 310617	Gujarat	21	IC283655	Gujarat
03	IC 310618	Gujarat	22	NR74	Gujarat
04	IC 310609	Gujarat	23	GUJ1	Gujarat
05	NR 127	Gujarat	24	NR61	Gujarat
06	IC 1112521	NBPGR, New Delhi	25	IC310906	Gujarat
07	IC 1112532	NBPGR, New Delhi	26	IC310903	Gujarat
08	IC 1112527	NBPGR, New Delhi	27	IC310904	Gujarat
09	IC 283671	Gujarat	28	IC310908	Gujarat
10	NMRM2	Gujarat	29	IC310614	Gujarat
11	IC 285630	Gujarat	30	IC112531	NBPGR, New Delhi
12	NR72	Gujarat	31	IC112517	NBPGR, New Delhi
13	IC 285626	Gujarat	32	K98	Gujarat
14	IC 310623	Gujarat	33	GUJ6	Gujarat
15	IC 310619	Gujarat	34	IC285629	Gujarat
16	MP1	Madhya Pradesh	35	N129	Gujarat
17	GUJ2	Gujarat	36	GUJ3	Gujarat
18	NR63	Gujarat	37	RAJ1	Rajasthan
19	IC283670	Gujarat	38	RAJ2	Rajasthan

Table 2: Details of ISSR Analysis in 38 Accessions of *Aloe barbadensis*

Sr. No.	Primer	5' -3' Sequence	Approx. Fragment Size (bp)	Total loci	Polymorphic loci	Percent of polymorphic loci	Resolving Power	Primer Index
01	$(CT)_8TG$	CTCTCTCTCTCTCTCTTG	420-1650	08	07	87.50	8.842	0.494
02	$(CA)_8AT$	CACACACACACACACAAT	590-1950	05	04	80	3.579	0.460
03	$(GTG)_3GC$	GTGGTGGTGGC	200-800	06	04	66.67	8.000	0.444
04	$(CA)_8GT$	CACACACACACACACAGT	250-1150	08	06	75	12.474	0.344
05	$(CT)_9G$	CTCTCTCTCTCTCTCTG	150-800	08	06	75	10.105	0.465
06	$(GA)_7RC$	GAGAGAGAGAGAGARC	400-1200	05	03	6O	7.211	0.402
Total				40	29	384.17	50.211	2.6094
Percentage					72.5			
Average				6.67	5	74.028	8.369	0.435

Table 3: Details of RAPD Analysis in 38 Accessions of *Aloe barbadensis* Using Different Primers

Sr. No.	Primer	5' -3' Sequence	Approx. Fragment Size (bp)	Total Number of loci	Number of Polymorphic loci	Percent of Polymorphic loci	Primer Index	Resolving Power
01	OPA09	GGGTAACGCC	250-2000	07	06	85.71	0.185	12.368
02	OPA20	GTTGCGATCC	250-1550	09	09	100	0.499	10.421
03	OPJ07	CCTCTCGACA	690-2980	07	05	71.42	0.306	4.053
04	OPJ09	TGAGCCTCAC	600-1350	05	04	80	0.447	6.632
05	OPJ10	AAGCCCGAGG	430-3000	05	04	80	0.468	7.526
06	OPJ11	ACTCCTGCGA	390-2550	11	11	100	0.436	14.947
07	OPJ12	GTCCCGTGGT	490-3000	04	03	75	0.383	7.421
08	OPJ13	CCACACTACC	600-2000	07	07	100	0.465	8.842
09	OPJ17	ACGCCAGTTC	500-3000	08	07	87.5	0.494	9.947
10	OPJ18	TGGTCGCAGA	190-1250	06	4	66.67	0.424	8.368
11	OPJ19	GGACACCACT	590-2000	05	4	80	0.473	3.842
12	OPJ20	AAGCGGCCTC	500-1200	03	2	66.67	0.500	4.105
13	OPN02	ACCAGGGGCA	490-1600	07	6	85.71	0.455	9.105
14	OPN16	AAGCGACCTG	190-1200	05	4	80	0.500	5.158
Total				89	76	983.68	6.035	118.77
Percentage					85.39			
Average				6.357	5.429	82.763	8.053	0.431

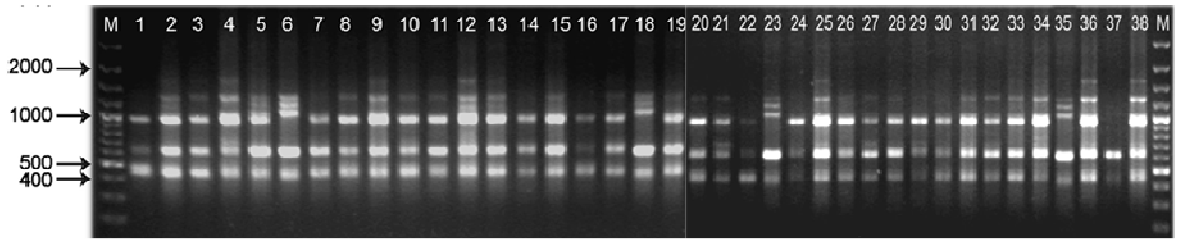


Figure 1: ISSR Profiling of Different Genotypes of *A. barbadensis* (Lane M- Gene Ruler 100bp Ladder, Lane 1-38-ISSR Primer [$(CA)_8$ AT]

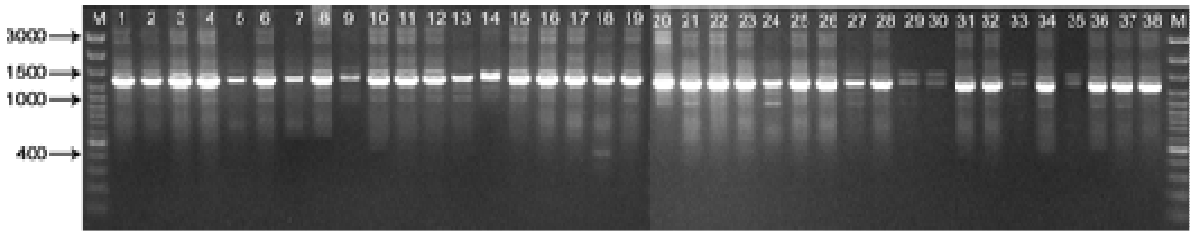
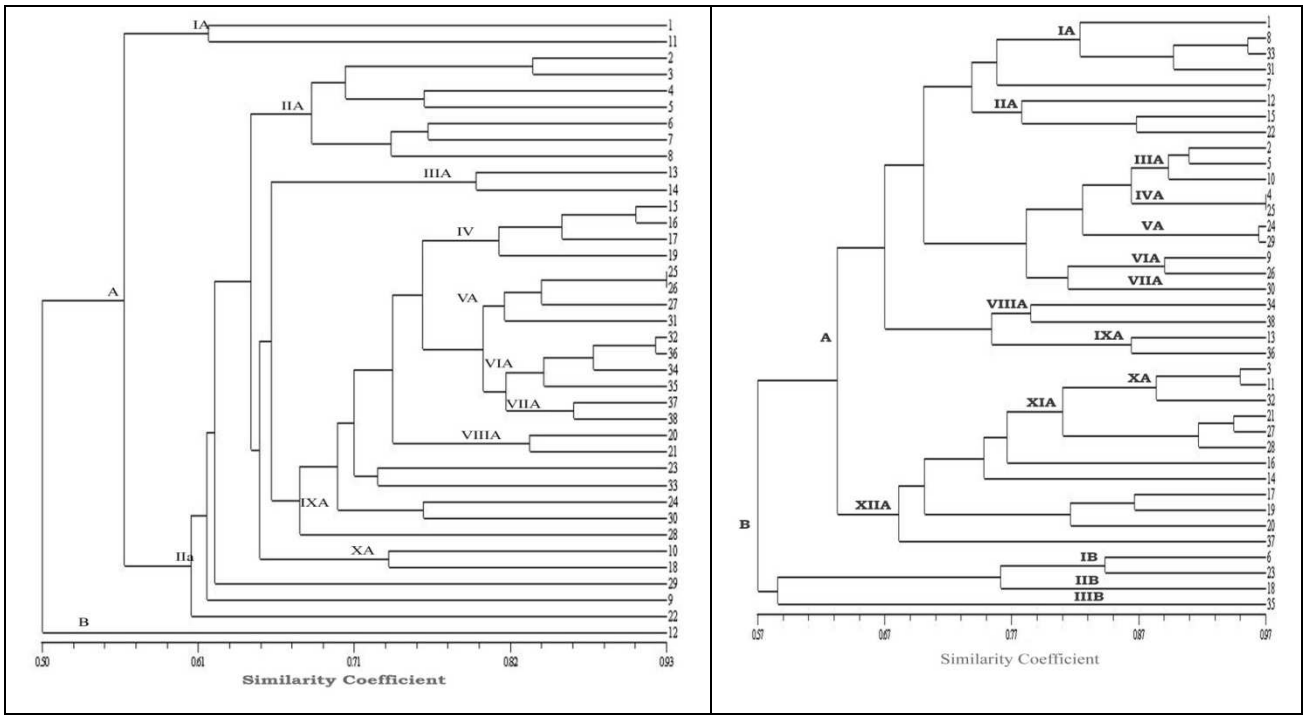
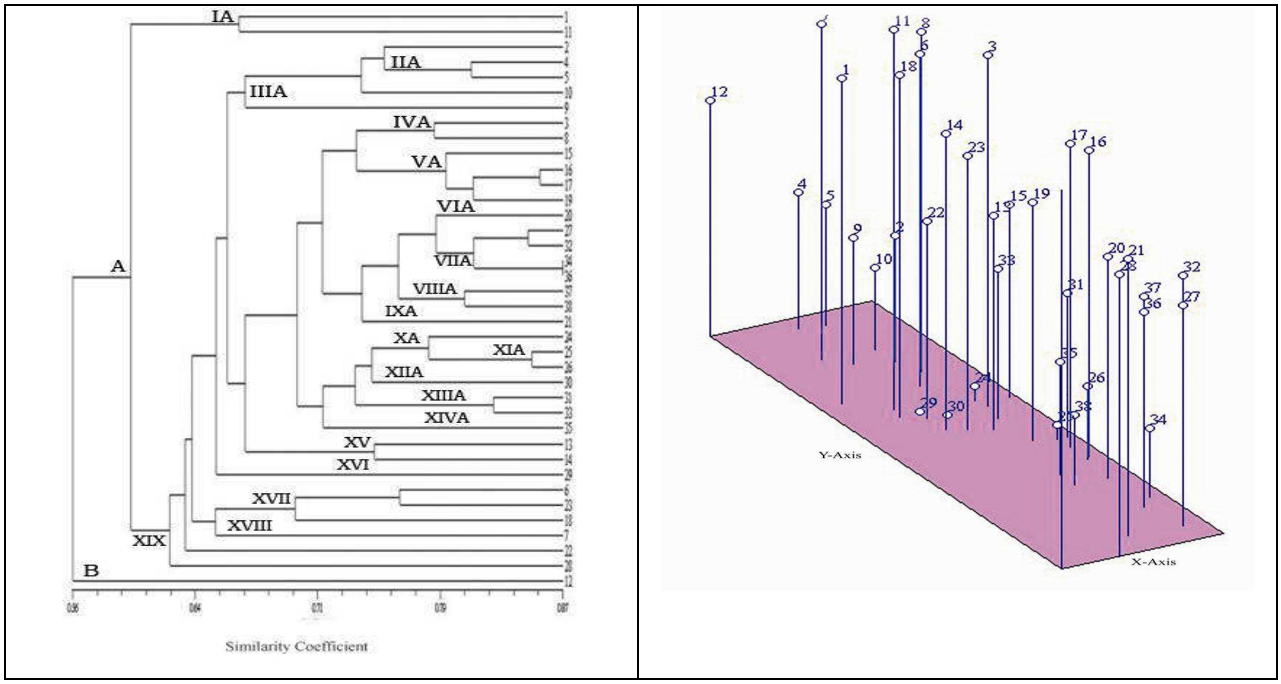


Figure 2: RAPD Profiling of Different Genotypes of *A. barbadensis* (Lane M- Gene Ruler 100bp Ladder, Lane 1-38- RAPD Primer OPJ10)



(a) **(b)**



(c) **(d)**

Figure 3: Dendrogram Generated Using UPGMA Analysis, Showing Relationships between *A. barbadensis* Genotypes (1-38) Using: (a) RAPD; (b) ISSR, and (c) Combined RAPD and ISSR Data, (d) Principal Co-Ordinate Analysis Based on Combined RAPD and ISSR Data. The *Scale Bar* Indicates the Similarity Index